Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000042

International filing date: 14 January 2005 (14.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/536,598
Filing date: 15 January 2004 (15.01.2004)

Date of receipt at the International Bureau: 13 April 2005 (13.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

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APPLICATION NUMBER: *60/536,598*
FILING DATE: *January 15, 2004*

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"Express Mail" mailing label number: EV 407362932 US
Date of Deposit: JANUARY 15, 2004

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By: _____
Name: JOHN JUNKERS

REQUEST FOR PROVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(c)

MAIL STOP PROVISIONAL PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is a request for filing a Provisional application for patent under 37 CFR § 1.53(c) entitled METHODS AND COMPOSITIONS FOR MODULATING THE ONSET OF LABOR by the following inventor(s):

Full Name Of Inventor	Family Name LYE	First Given Name STEPHEN	Second Given Name
Residence & Citizenship	City TORONTO	State or Foreign Country ONTARIO	Country of Citizenship UNITED KINGDOM
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☒ Enclosed is the Provisional application for patent as follows: 20 pages of specification, and 0 sheets of drawings.

☐ Small entity status is claimed pursuant to 37 CFR 1.27.

☒ Payment of Provisional filing fee under 37 C.F.R. § 1.16(k) :
- ☒ Attached is a check in the amount of $ 80.00.
- ☐ Please charge Deposit Account No. 13-2725.
- ☐ PAYMENT OF THE FILING FEE IS BEING DEFERRED.

☒ The Commissioner is hereby authorized to charge any additional fees as set forth in 37 CFR §§ 1.16 to 1.18 which may be required by this paper or credit any overpayment to Account No. 13-2725.

☐ Enclosed is an Assignment of the invention to , Recordation Form Cover Sheet and a check for $ to cover the Recordation Fee.

☐ Also Enclosed:

☐ The invention was made by the following agency of the United States Government or under a contract with the following agency of the United States Government:

☒ Address all future communications to the **Attention of Douglas P. Mueller** (may only be completed by attorney or agent of record) at the address below.

☒ A return postcard is enclosed.

Respectfully submitted,

23552
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Title: **Methods and Compositions for Modulating the Onset of Labor**

FIELD OF THE INVENTION

The invention relates to methods and compositions for diagnosing, inducing, and preventing the onset of labor.

BACKGROUND OF THE INVENTION

One of most significant problems in obstetrics is the management of pre-term labor. A significant number of the pregnancies progressing past 20 weeks of gestation experience premature labor and delivery, which is a leading cause of infant deaths and long-term neurological handicaps, including cerebral palsy, blindness, deafness, and developmental defects. To date the efforts to reduce the incidence of pre-term labor have been unsuccessful. This is attributed to a number of factors including the difficulties in identifying pregnancies at risk for preterm labor, the lack of reliable diagnosis of pre-term labor, and the inability to effectively intervene.

Summary of the Invention

Applicant has identified novel progesterone receptor (PR)-interacting proteins that block the progesterone receptor signaling pathway at term in human pregnancy. One of the PR-interacting proteins was identified as PSF, a RNA splicing factor. The interaction between PR and PSF was confirmed by *in vivo* and *in vitro* protein interaction assays. PSF was found to interact with both the PRA and PRB isoforms. The interaction domains were found to be located in the AF3 and DNA binding domain of PR and the RRM (RNA recognizing motif) of PSF. Co-transfection of PSF into myometrial cells resulted in decreased transcriptional activity of PRB, but not of ERα or ERβ. Over-expression of PSF in 293T cells reduced PR protein levels, an effect that could be rescued by the proteosomal inhibitor, MG132. A very low level of expression of PSF was found in the rat myometrium during pregnancy but a dramatic increase was found near term with maximal levels at the onset of labour. PSF's interaction with the DNA binding domain of PR blocks PR-mediated transcriptional activity. PSF targets PR for degradation through the 26S proteosome pathway. Together with the increased myometrial expression of PSF at term, these data indicate that PSF acts to induce a functional withdrawal of progesterone and initiate labour.

Broadly stated the present invention relates to a method for detecting, preventing, or inducing the onset of labor by modulating PSF in a subject.

In an aspect the invention provides methods for identifying the onset of labor in a subject comprising detecting PSF in a sample from the subject. In an embodiment of the

diagnostic method of the invention, a method is provided for diagnosing increased risk of pre-term labor in a subject comprising detecting PSF in a sample from the subject.

The invention also broadly contemplates a method for regulating the onset of labor in a subject comprising inhibiting or stimulating PSF. In an embodiment of the invention, a method is provided for inhibiting the onset of labor in a subject comprising administering to the subject an effective amount of an inhibitor of PSF. In an embodiment, a method is provided for controlling pre-term labor sufficiently to extend pregnancy in a subject to as close to full term as possible comprising administering to the subject an effective amount of an inhibitor of PSF.

The invention provides a method of preventing premature labor in a subject susceptible thereto, comprising administration of a labor preventive amount of an inhibitor of PSF to the subject.

In an embodiment of the invention a method is provided for treating a female suffering from, or who may be susceptible to pre-term labor comprising administering therapeutically effective dosages of an inhibitor of PSF. A therapeutically effective dosage is an amount of an inhibitor of PSF effective to maintain progesterone receptor levels thus inhibiting the onset of labor.

The invention also provides a method for reducing the risk of pre-term labor in a subject at risk therefore comprising administration of a labor preventive amount of an inhibitor of PSF to the subject.

The methods and compositions of the invention may also be used to stop labor preparatory to Cesarean delivery. Thus, the invention relates to a method for stopping labor preparatory to Cesarean delivery in a subject in need of such treatment comprising administration of a therapeutically effective amount of an inhibitor of PSF to the subject.

The present invention is also directed to a method for controlling the timing of parturition in farm animals so that delivery of the neonates occurs during the daytime and thus can be readily monitored. A PSF inhibitor is administered to the mother on the evening before the expected delivery to delay parturition so that the delivery occurs during the daylight hours. Delaying the timing of parturition enables proper monitoring of the delivery and neonates, resulting in increased survival rates of the newborn.

In another embodiment of the invention, a method is provided for inducing labor in a subject comprising administering an effective amount of PSF. In a preferred embodiment, a method is provided for inducing labor in a subject comprising administering therapeutically

effective dosages of an inhibitor of PSF. An amount is administered which is effective to up regulate or stimulate PSF in the subject.

The invention also relates to a composition adapted for regulating the onset of labor comprising a substance which inhibits or stimulates PSF, in an amount effective to inhibit or stimulate the onset of labor, and an appropriate carrier, diluent, or excipient. In an embodiment of the invention, a composition is provided for treating a woman suffering from, or who may be susceptible to pre-term labor, comprising a therapeutically effective amount of an inhibitor of PSF, and a carrier, diluent, or excipient. In another embodiment of the invention, a composition is provided for inducing labor in a subject comprising a therapeutically effective amount of PSF, and a carrier, diluent, or excipient.

The invention further relates to the use of a PSF inhibitor or PSF for the manufacture of a medicament useful in modulating the onset of labor. The invention still further relates to the use of a PSF inhibitor for the manufacture of a medicament useful for preventing preterm or premature labor, reducing the risk of premature labor, stopping labor preparatory to Cesarean delivery, and controlling the timing of parturition in farm animals.

The invention further relates to a method of selecting a substance that regulates the onset of labor comprising assaying for a substance that inhibits or stimulates PSF. The substances may be used in the methods of the invention to regulate the onset of labor.

The invention also relates to kits for carrying out the methods of the invention.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention there may be employed conventional biochemistry, enzymology, molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See for example, Sambrook, Fritsch, & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization B.D. Hames & S.J. Higgins eds. (1985); Transcription and Translation B.D. Hames & S.J. Higgins eds

(1984); Animal Cell Culture R.I. Freshney, ed. (1986); Immobilized Cells and enzymes IRL Press, (1986); and B. Perbal, A Practical Guide to Molecular Cloning (1984).

"PSF" refers to polypyrimidine tract-binding protein-associated splicing factor [Patton, J.G., Porro, E.B., Galceran, J., Tempst, P. and Nadal-Ginard, B. TITLE Cloning and characterization of PSF, a novel pre-mRNA splicing factor JOURNAL Genes Dev. 7 (3), 393-406 (1993)] including human PSF (GenBank NP_005057 and P23246) and mouse PSF (GenBank NP 076092). "PSF" includes the wild type protein, or part thereof, or a mutant, variant or homolog of such a protein.

The term "wild type" refers to a polypeptide having a primary amino acid sequence that is identical with the native protein (for example, the human or mouse protein). The term "mutant" refers to a polypeptide having a primary amino acid sequence which differs from the wild type sequence by one or more amino acid additions, substitutions or deletions. Preferably, the mutant has at least 90% sequence identity with the wild type sequence. Preferably, the mutant has 20 mutations or less over the whole wild-type sequence. More preferably the mutant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

The term "variant" refers to a naturally occurring polypeptide that differs from a wild-type sequence. A variant may be found within the same species (i.e. if there is more than one isoform of the protein) or may be found within a different species. Preferably the variant has at least 90% sequence identity with the wild type sequence. Preferably, the variant has 20 mutations or less over the whole wild-type sequence. More preferably, the variant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

The term "part" indicates that the polypeptide comprises a fraction of the wild-type amino acid sequence. It may comprise one or more large contiguous sections of sequence or a plurality of small sections. The polypeptide may also comprise other elements of sequence, for example, it may be a fusion protein with another protein (such as one which aids isolation or crystallization of the polypeptide). Preferably the polypeptide comprises at least 50%, more preferably at least 65%, most preferably at least 80% of the wild-type sequence.

The term "homolog" means a polypeptide having a degree of homology with the wild-type amino acid sequence. The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology. In an embodiment of the invention a PSF protein is substantially homologous to a wild type protein. A sequence that is "substantially homologous" refers to a partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid. Inhibition of

hybridization of a completely complementary sequence to the target sequence may be examined using a hybridization assay (e.g. Southern or northern blot, solution hybridization, etc.) under conditions of reduced stringency. A sequence that is substantially homologous or a hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of reduced stringency. However, conditions of reduced stringency can be such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested using a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% homology or identity). The substantially homologous sequence or probe will not hybridize to the second non-complementary target sequence in the absence of non-specific binding.

A sequence of a PSF protein contemplated by the invention may have at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity. The phrases "percent identity" or "% identity" refer to the percentage comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically using for example the MegAlign program (DNASTAR, Inc., Madison Wis.). The MegAlign program can create alignments between two or more sequences according to different methods, e.g., the Clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) Percent identity between nucleic acid sequences can also be determined by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) In addition, identity between sequences can be determined by other methods known in the art, e.g., by varying hybridization conditions.

PSF proteins include chimeric or fusion proteins. A "chimeric protein" or "fusion protein" comprises all or part (preferably biologically active) of a PSF polypeptide operably linked to a heterologous polypeptide (i.e., a polypeptide other than a PSF polypeptide). Within the fusion protein, the term "operably linked" is intended to indicate that a PSF polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of a PSF polypeptide. A useful fusion protein is a GST fusion protein in which a PSF polypeptide is fused to the C-terminus of GST sequences. Another example of a fusion protein is an immunoglobulin fusion protein in which all or part of a PSF polypeptide is fused to sequences derived from a member of the immunoglobulin protein family. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

The terms "sample", "biological sample", and the like mean a material known or suspected of expressing or containing PSF associated with onset of labor. The test sample can be used directly as obtained from the source or following a pretreatment to modify the character of the sample. The sample can be derived from any biological source, such as tissues, extracts, or cell cultures, including cells, cell lysates, and physiological fluids, such as, for example, whole blood, plasma, serum, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, synovial fluid, peritoneal fluid and the like.

The sample can be obtained from animals, preferably mammals, most preferably humans. The sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, the addition of reagents, and the like. Proteins may be isolated from the samples and utilized in the methods of the invention.

In embodiments of the invention the sample is a mammalian tissue sample. In another embodiment the sample is a human physiological fluid. In a particular embodiment, the sample is human serum.

The terms "subject", "individual" or "patient" refer to a warm-blooded animal such as a mammal, which is afflicted with or suspected of having pre-term labor or other condition as described herein. In particular, the terms refer to a human.

Diagnostic Methods

As hereinbefore mentioned, the present invention provides a method for diagnosing or monitoring in a subject a condition requiring regulation of labor comprising detecting PSF in a sample from the subject. In an embodiment of the diagnostic method of the invention, a method is provided for diagnosing increased risk of pre-term labor in a subject comprising detecting PSF in a sample from the subject.

PSF may be detected in a variety of samples from a patient. Examples of suitable samples include cells (e.g. fetal or maternal); and, fluids (fetal or maternal), including for example, serum, plasma, amniotic fluid, saliva, and conditioned medium from fetal or maternal cells.

PSF may be detected using a substance which directly or indirectly interacts with the PSF For example, antibodies specific for PSF may be used to diagnose and monitor a condition requiring regulation of labor. A method of the invention using antibodies may utilize Countercurrent Immuno-Electrophoresis (CIEP), Radioimmunoassays, Radioimmunoprecipitations, and Enzyme-Linked Immuno-Sorbent Assays (ELISA), Dot

Blot assays, Inhibition or Competition assays and sandwich assays (see U.S. Patent Nos. 4,376,110 and 4,486,530).

Antibodies used in the methods of the invention include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, and $F(ab')_2$ and recombinantly produced binding partners. Polyclonal antibodies may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, or rats. Monoclonal antibodies may also be readily generated using conventional techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993 which are incorporated herein by reference; see also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference). Binding partners may be constructed utilizing recombinant DNA techniques to incorporate the variable regions of a gene which encodes a specifically binding antibody (See Bird et al., Science 242:423-426, 1988). Antibodies may also be obtained from commercial sources.

The presence of PSF may be determined by measuring the binding of the PSF to molecules (or parts thereof) which are known to interact with PSF including but not limited to the progesterone receptor. Progesterone receptor includes the A and B forms of the receptor, and parts thereof including the AF3 and DNA binding domain (See US Patent No. 5,439,796, Beato, M., Cell 5:335-344 (1989) Green et al., Nature 328:134-139 (1986); Hollenberg et al., Nature 318:635-641 (1985); Arriza et al., Science 237.:268-275 (1987); Mishraki et al., Biochem. Biophys. Res. Comm. 143:740-748 (1987); Lubahn et al., Science 240:327-330 (1988); Chang et al., Science 240:324-326 (1988)).

In a particular aspect, peptides derived from sites on the progesterone receptor which bind to PSF may be used (e.g. AF3 and DNA binding domain). A peptide derived from a specific site on the receptor may encompass the amino acid sequence of a naturally occurring binding site, any portion of that binding site, or other molecular entity that functions to bind an associated molecule. A peptide derived from such a site will interact directly or indirectly with an associated molecule in such a way as to mimic the native binding site. Such peptides may include competitive inhibitors, enhancers, peptide mimetics, and the like as discussed below. The molecules that interact with the PSF are referred to herein as "Binding Compounds".

The antibodies specific for PSF or the Binding Compounds may be labeled using conventional methods with various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive materials include radioactive phosphorous ^{32}P, iodine I^{125}, I^{131} or tritium.

An antibody to PSF or a Binding Compound may also be indirectly labeled with a ligand binding partner. For example, the antibodies, or Binding Compound may be conjugated to one partner of a ligand binding pair, and the PSF may be coupled to the other partner of the ligand binding pair. Representative examples include avidin-biotin, and riboflavin-riboflavin binding protein. In an embodiment the antibodies are biotinylated. Methods for conjugating the antibodies discussed above with the ligand binding partner may be readily accomplished by one of ordinary skill in the art (see Wilchek and Bayer, "The Avidin-Biotin Complex in Bioanalytical Applications,"Anal. Biochem. 171:1-32, 1988).

The antibodies or Binding Compounds used in the method of the invention may be insolubilized. For example, the antibodies or Binding Compounds may be bound to a suitable carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The carrier may be in the shape of, for example, a tube, test plate, beads, disc, sphere etc. The insolubilized compound or antibodies may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

Indirect methods may also be employed in which a primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against PSF. By way of example, if the antibody having specificity against PSF is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labeled with a detectable substance as described herein.

PSF can also be assayed in a sample using nucleotide probes to detect nucleic acid molecules encoding a PSF. Suitable probes include nucleic acid molecules based on nucleic acid sequences encoding PSF. A nucleotide probe may be labeled with a detectable substance

such as a radioactive label which provides for an adequate signal and has sufficient half-life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and luminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.).

A nucleic acid molecule encoding PSF can also be detected by selective amplification of the nucleic acid molecules using polymerase chain reaction (PCR) methods. Synthetic oligonucleotide primers can be constructed from the sequences of PSF using conventional methods. A nucleic acid can be amplified in a sample using these oligonucleotide primers and standard PCR amplification techniques.

In an embodiment of the invention, a method is provided for diagnosing increased risk of pre-term labor in a subject comprising detecting PSF or a complex of PSF and progesterone receptor in a sample, and in particular using antibodies specific for PSF. Levels of PSF or complexes thereof may be measured prior to or during the onset of labor. If the levels are significantly increased as compared to levels typical for women who do not suffer from pre-term labor, the patient is diagnosed as having an increased risk of pre-term labor. Levels above those typical for women who do not suffer from pre-term labor may be suspect and further monitoring and measurement PSF may be appropriate. The information from the diagnostic method may be used to identify subjects who may benefit from a course of treatment, such as treatment via administration of inhibitors of PSF as discussed herein.

The invention also relates to kits for carrying out the methods of the invention. The kits comprise instructions, negative and positive controls, and means for direct or indirect measurement of PSF or complexes of same.

Regulation of Labor Onset in a Subject

The invention also provides a method of regulating the onset of labor comprising directly or indirectly inhibiting or stimulating PSF.

In an embodiment of the invention, a method is provided for inhibiting the onset of pre-term labor in a subject comprising administering an effective amount of a substance

which is an inhibitor of PSF. In particular, methods are provided for treating a women suffering from or who may be susceptible to pre-term labor.

In another embodiment of the invention, a method is providing for inducing labor in a subject comprising administering an effective amount of PSF.

Substances that regulate the onset of labor can be selected by assaying for a substance that inhibits or stimulates the activity of PAF. A substance that regulates the onset of labor can also be identified based on its ability to specifically interfere or stimulate the interaction of PSF and progesterone receptor.

Therefore, a method is provided for evaluating a compound for its ability to regulate the onset of labor comprising the steps of:

(a) reacting PSF or a part thereof that binds to a progesterone receptor (e.g. RNA recognizing motif) with a progesterone receptor or a part thereof that binds to PSF (e.g. AF3 and DNA binding domain), and a test substance; and

(b) comparing to a control in the absence of the test substance to determine the effect of the substance.

In particular, a method is provided for identifying a substance that regulates the onset of labor comprising the steps of:

(a) reacting PSF or a part thereof that binds to a progesterone receptor (e.g. RNA recognizing motif) with a progesterone receptor or a part thereof that binds to PSF (e.g. AF3 and DNA binding domain), and a test substance, under conditions which permit the formation of PSF-receptor complexes, and

(b) assaying for complexes, for free substance, for non-complexed PSF or receptor, or for activation of the receptor.

The substance may stimulate or inhibit the interaction of PSF or a part thereof that binds the receptor, and the receptor.

Activation of the receptor may be assayed by measuring phosphorylation of the receptor, by assaying for a biological affect on a cell, or by measuring biochemical markers.

The substances identified using the methods of the invention include but are not limited to peptides such as soluble peptides including Ig-tailed fusion peptides, members of random peptide libraries and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries), antibodies [e.g. polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, single chain antibodies, fragments, (e.g.

Fab, $F(ab)_2$, and Fab expression library fragments, and epitope-binding fragments thereof)], and small organic or inorganic molecules. The substance may be an endogenous physiological compound or it may be a natural or synthetic compound. The substance may be a PSF-receptor complex, which competitively inhibits the binding of PSF to its natural receptor. The invention contemplates isolated PSF-progesterone receptor complexes and their use in regulating the onset of labor.

The substances may be peptides derived from the binding sites of PSF and progesterone receptor, or a complex of PSF and progesterone receptor. A peptide derived from a specific binding site may encompass the amino acid sequence of a naturally occurring binding site, any portion of that binding site, or other molecular entity that functions to bind an associated molecule. A peptide derived from such a binding site will interact directly or indirectly with an associated molecule in such a way as to mimic the native binding domain. Such peptides may include competitive inhibitors, enhancers, peptide mimetics, and the like. All of these peptides as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides may be used for purposes of the present invention.

"Peptide mimetics" are structures which serve as substitutes for peptides in interactions between molecules (See Morgan et al (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures which may or may not contain amino acids and/or peptide bonds but retain the structural and functional features of a peptide, or enhancer or inhibitor of the invention. Peptide mimetics also include peptoids, oligopeptoids (Simon et al (1972) Proc. Natl. Acad, Sci USA 89:9367); and peptide libraries containing peptides of a designed length representing all possible sequences of amino acids corresponding to a peptide of the invention.

Peptides may be synthesized by conventional techniques. For example, the peptides may be synthesized by chemical synthesis using solid phase peptide synthesis. These methods employ either solid or solution phase synthesis methods (see for example, J.M. Stewart, and J.D. Young, Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford III. (1984) and G. Barany and R.B. Merrifield, The Peptides: Analysis Synthesis, Biology editors E. Gross and J. Meienhofer Vol. 2 Academic Press, New York, 1980, pp. 3-254 for solid phase synthesis techniques; and M Bodansky, Principles of Peptide Synthesis, Springer-Verlag, Berlin 1984, and E. Gross and J. Meienhofer, Eds., The Peptides: Analysis, Synthesis, Biology, supra, Vol 1, for classical solution synthesis.)

Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of inhibitor peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

A substance that regulates the onset of labor may be a molecule which interferes with the transcription and/or translation of PSF. For example, the sequence of a nucleic acid molecule encoding PSF, a complex thereof, or fragments thereof, may be inverted relative to its normal presentation for transcription to produce an antisense nucleic acid molecule. An antisense nucleic acid molecule may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art.

A substance that regulates the onset of labor may be an apatmer. An aptamer includes a DNA or RNA molecule that binds to nucleic acids and proteins. An aptamer that binds to a PSF (or binding domain thereof) can be produced using conventional techniques, without undue experimentation. [For example, see the following publications describing *in vitro* selection of aptamers: Klug et al., Mol. Biol. Reports 20:97-107 (1994); Wallis et al., Chem. Biol. 2:543-552 (1995); Ellington, Curr. Biol. 4:427-429 (1994); Lato et al., Chem. Biol. 2:291-303 (1995); Conrad et al., Mol. Div. 1:69-78 (1995); and Uphoff et al., Curr. Opin. Struct. Biol. 6:281-287 (1996)].

The treatment methods and compositions described herein may use substances that are known inhibitors of PSF.

The utility of a selected inhibitor or stimulator may be confirmed in experimental model systems.

In an embodiment of the invention a method is provided for treating a woman suffering from, or who may be susceptible to pre-term labor comprising administering therapeutically effective dosages of an inhibitor of PSF, or a substance identified in

accordance with the methods of the invention. Treatment with the inhibitor may commence prior to or after onset of labor, and may continue until measured PSF levels are within the normal range. For the purposes of the present invention normal PSF levels are defined as those levels typical for pregnant women who do not suffer from pre-term labor. Treatment with the inhibitor is discontinued after PSF levels are within normal range, and before any adverse effects of administration of the inhibitor are observed. Inhibition may be reversed for example by treatment with a proteosomal inhibitor.

One or more inhibitors or one or more stimulators of PSF, or substances selected in accordance with the methods of the invention including Binding Compounds, may be incorporated into a composition adapted for regulating the onset of labor. In an embodiment of the invention, a composition is provided for treating a woman suffering from, or who may be susceptible to pre-term labor, comprising a therapeutically effective amount of an inhibitor of PSF or substance selected in accordance with the methods of the invention including Binding Compounds, and a carrier, diluent, or excipient.

The compositions of the invention contain at least one inhibitor or stimulator of PSF or substance identified in accordance with the methods of the invention, alone or together with other active substances. Such compositions can be for oral, rectal, intravaginal, topical, parenteral (including subcutaneous, intramuscular and intravenous administration), or local use. They can therefore be in solid or semisolid form, for example pills, tablets, and capsules.

The composition of the invention can be intended for administration to subjects such as humans or animals. The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle, carrier or diluent. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

The compositions of the invention may be administered together with or prior to administration of other agents that have been found to affect pre-term labor, including 5 alpha-reductase inhibitors (US Patent No. 5,872,126 to Cukierski et al.), and tocolytic agents used in the treatment of pre-term labor such as beta-adrenergic agonists, magnesium sulfate, ethanol, oxytocin antagonists, calcium transport blockers, prostaglandin synthesis inhibitors, nitric oxide donors, phosphodiesterase inhibitors, and progestins. The individual components can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms.

The compositions and other agents may be administered through any known means. Systemic administration, such as intravenous or subcutaneous administration is preferred. A therapeutically effective amount of an active ingredient e.g. inhibitor is an amount effective to elicit the desired therapeutic response but insufficient to cause a toxic reaction. The dosage for the compositions is determined by the attending physician taking into account factors such as the condition, body weight, diet of the subject, and the time of administration.

For example, a therapeutically effective dose of an inhibitor, e.g. an amount sufficient to lower levels of PSF to normal levels, may be about 1 to 200 μg/kg/day. The method of the invention may involve a series of administrations of the composition. Such a series may take place over a period of 7 to about 21 days and one or more series may be administered. The composition may be administered initially at the low end of the dosage range and the dose will be increased incrementally over a preselected time course.

An inhibitor or stimulator of PSF or substance identified in accordance with the methods of the invention may be administered by gene therapy techniques using genetically modified cells or by directly introducing genes encoding the inhibitors or stimulators of PSF, or substances into cells *in vivo*. Cells may be transformed or transfected with a recombinant vector (e.g. retroviral vectors, adenoviral vectors and DNA virus vectors). Genes encoding inhibitors or stimulators, or substances may be introduced into cells of a subject *in vivo* using physical techniques such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes. Antisense molecules may also be introduced *in vivo* using these conventional methods.

The following non-limiting examples are illustrative of the present invention:

EXAMPLE 1

Progesterone is an essential regulator of the reproductive events associated with the establishment and maintenance of pregnancy through its ligand-activated progesterone receptor (PR). Progesterone actions include the suppression of genes encoding contraction-associated proteins (CAPs, e.g. oxytocin receptor, prostaglandin receptor, connexin43) that are required for myometrial activation and the onset of labor. In the human, progesterone levels remain elevated through labour and even in species where progesterone levels fall at term, concentrations are likely sufficiently high to inhibit CAP gene expression. This suggests there must be an active mechanism for inducing a functional withdrawal of progesterone at term. The objective of this study was to identify novel PR-interacting proteins that might block the PR signaling pathway at term in human pregnancy. GST-PR fusion proteins were used to "pulldown" interacting proteins in myometrial cell homogenates

and the identity of these proteins was determined by MALDI-TOF Mass Spectrometry. One of the PR-interacting proteins was identified as PSF, a previously recognized RNA splicing factor. The interaction between PR and PSF was confirmed by *in vivo* (mammalian two-hybrid system) and *in vitro* (GST-pull down assay using purified proteins) protein interaction assays. PSF was found to interact with both the PRA and PRB isoforms. The interaction domains were found to be located in the AF3 and DNA binding domain of PR and the RRM (RNA recognizing motif) of PSF. Co-transfection of PSF into myometrial cells resulted in decreased transcriptional activity of PRB, but not of ERα or ERβ. Over-expression of PSF in 293T cells reduced PR protein levels, an effect that could be rescued by the proteosomal inhibitor, MG132. Of significance, we demonstrated a very low level of expression of PSF in the rat myometrium during pregnancy but a dramatic increase near term with maximal levels at the onset of labour. Thus, we have defined novel functions of PSF beyond its actions as a pre-mRNA splicing factor. PSF's interaction with the DNA binding domain of PR blocks PR-mediated transcriptional activity. PSF targets PR for degradation through the 26S proteosome pathway, possibly by interacting with ubiquitin ligases. Together with the increased myometrial expression of PSF at term, these data suggest that PSF may act to induce of a functional withdrawal of progesterone and initiate labour.

The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. All publications, patents and patent applications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, methodologies etc. which are reported therein which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

We Claim:

1. A method for preventing and/or treating pre-term labor comprising modulating PSF.
2. A method for diagnosing in a subject a condition requiring regulation of the onset of labor comprising detecting PSF in a sample from the subject.
3. A method for diagnosing increased risk of pre-term labor in a subject comprising detecting PSF in a sample from the subject.
4. A method as claimed in claim 3 which comprises (a) collecting a sample from the subject; (b) measuring the levels of PSF in the sample; and (c) comparing the levels of PSF in the sample to the levels in females with normal pregnancies.
5. A method of claim 4 wherein significantly increased levels in the sample compared to levels in samples from women who do not suffer from pre-term labor is indicative of an increased risk of pre-term labor.
6. A method of regulating the onset of labor comprising inhibiting or stimulating PSF.
7. A method for preventing or reducing pre-term labor in a subject comprising administering an effective amount of an inhibitor PSF.
8. A method for treating a female suffering from, or who may be susceptible to pre-term labor comprising administering therapeutically effective dosages of an inhibitor of PSF.
10. A method for inducing labor in a subject comprising administering therapeutically effective dosages of PSF.
11. A method for evaluating a compound for its ability to regulate the onset of labor comprising the steps of:
    (a) reacting PSF and progesterone receptor, and a test substance; and
    (b) comparing to a control in the absence of the substance to determine if the substance stimulates or inhibits the binding of PSF to the receptor and thereby regulates the onset of labor.
12. A method for evaluating a substance for its ability to regulate the onset of labor comprising the steps of:
    (a) reacting PSF and a progesterone receptor and a test substance, wherein the PAF and receptor bind to form a complex; and
    (b) comparing to a control in the absence of the substance to determine if the substance stimulates or inhibits the binding of PSF to the receptor and thereby regulates the onset of labor.

13. A complex comprising PSF-progesterone receptor.

ABSTRACT OF THE DISCLOSURE

Methods and compositions are provided for detecting, and modulating or regulating the onset of labor. The methods involve measuring levels of PSF, and treating with PSF or inhibitors of PSF.

Sequence Listing

P23246

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